WEST Search History

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DATE: Friday, April 28, 2006

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		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=OR	
☐	L1	botulin or botulinum or botulism or bont or botx or bottox or bot-tox or btxa or btx-a or bnt or bnta or bnt-a	9947
☐	L2	L1 and (peptide or polypeptide or poly-peptide or epitope or $tope or fragment).clm.	1913
☐	L3	L2 and l1.ti,ab,clm.	222
☐	L4	L3 and (nterminal or n-terminal or hn or (h near n) or h-n or 785 or 981 or 1121 or 1051 or 1275).clm.	19
☐	L5	l1 near25 epitop$	42

END OF SEARCH HISTORY

DOCUMENT-IDENTIFIER: US 6429189 B1
TITLE: Cytotoxin (non-neurotoxin) for the treatment of human headache disorders and inflammatory diseases

Detailed Description Text (32):
FIG. 1c\Note the pattern of erythema on the neck of this patient with cervcial dystonia. This patient has been well documented to exhibit circulating neutralizing antibodies to the neuromuscular paralyzing effects of the immunotype A Clostridium botulinum toxin. No muscular atrophy is seen in injected muscle yet there is a block in the erythema surrounding the injection site in a dimension equivalent to the diffusion potential for 20-40 LD 50 units of botulinum toxin. As neutralizing antibodies to the neuromuscular effect of botulinum did not block this anti-pain and anti-inflammatory effect, the anti-inflammatory effect may be the result of a different immuno-reactive epitope on the botulinum toxin proteins.

Previous Doc Next Doc Go to Doc#

Search notes

US006429189B1

(12) **United States Patent**
Borodic

(10) **Patent No.: US 6,429,189 B1**
(45) **Date of Patent: Aug. 6, 2002**

(54) **CYTOTOXIN (NON-NEUROTOXIN) FOR THE TREATMENT OF HUMAN HEADACHE DISORDERS AND INFLAMMATORY DISEASES**

(75) Inventor: **Gary E. Borodic**, Canton, MA (US)

(73) Assignee: **Botulinum Toxin Research Associates, Inc.**, Qunicy, MA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/458,784**

(22) Filed: **Dec. 10, 1999**

(51) **Int. Cl.**[7] **A01N 37/18**

(52) **U.S. Cl.** **514/2**; 514/2; 514/14; 514/825; 514/885; 424/810; 424/443; 424/430.1; 424/282.1; 530/350; 530/387.1; 530/389.5; 435/6; 435/842

(58) **Field of Search** 514/14, 2, 825, 514/885; 424/282.1, 810, 443, 130.1; 530/387.1, 350, 389.5

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,053,005 A	10/1991	Borodic	604/51
5,183,462 A	2/1993	Borodic	604/51
5,298,019 A	3/1994	Borodic	604/51
5,401,243 A	3/1995	Borodic	604/51
5,562,907 A	10/1996	Arnon	514/2
5,696,077 A	12/1997	Johnson et al.	514/2
5,714,468 A	2/1998	Binder	514/14
5,837,265 A	11/1998	Montal et al.	424/239.1
6,063,768 A *	5/2000	First	514/14
6,113,915 A	9/2000	Aoki et al.	424/236.1

OTHER PUBLICATIONS

Ohishi et al., Infect. Immun., vol. 31, No. 3, pp.890–895, 1981.*

Acquadro M et al. Treatment of myofascial pain with botulinum A toxin. Anesthesiology 3/94;80(3): 705–6.

Amann R et al. Intraplantar injection of nerve growth factor into the rat hind paw: local edema and effects on thermal nociceptive threshold. Pain, 1995; 64: 323–329.

Ashikaga T et al. Multiple daily insulin injections in the treatment of diabetic retinopathy. The Job study revisited. Diabetes, 5/78; 27(5): 592–6.

Bischoff SC et al. Effect of Nerve Growth Factor on the Release of Inflammatory Medators by Mature Human Basophils. Blood, May 15, 1992; 79(10): 2663–2669.

Borodic G et al. Contralateral injections of botulinum A toxin for the treatment of hemifacial spasm to achieve increased facial symmetry. Plast Reconstr Surg 12/92; 90(6): 972–7.

Borodic G Myasthenic crisis after botulinum toxin. Lancet, Dec. 5, 1998; 352(9143): 1832.

Borodic G et al. Botulinum toxin therapy, immunologic resistance, and problems with available materials. Neurology 1/96; 46(1): 26–9.

Borodic G et al. Antibodies to botulinum toxin. Neurology 1/95; 45(1): 204.

Borodic G Therapeutic botulinum toxin. Lancet Nov. 12, 1994; 344(8933): 1370.

Borodic G et al. New concepts in botulinum toxin therapy. Drug Saf 9/94; 11(3): 145–52.

Borodic G et al. Antibodies to bowline toxin. Ophthalmology 7/94; 11(3): 1158–9.

Borodic G et al. Histologic assessment of dose–related diffusion and muscle fiber response after therapeutic botulinum A toxin injection. Movement Disorders 1994; 9(1): 31–39.

Borodic G et al. Botulinum A toxin for treatment of aberrant facial nerve rgeneration. Plast Reconstr Surg 5/93; 91(6): 1042–5.

Borodic G et al. Botulinum B toxin as an alternative to botulinum A toxin: a histologic study. Ophthal Plast Reconstr Surg 1993; 9(3): 182–90.

Borodic G et al. Effects of repeated botulinum toxin injections on orbicularis oculi muscle. J Clin Neuroophthalmol 6/92; 12(2):121–7.

Borodic G et al. Botulinum A toxin for spasmodic torticollis: multiple vs single injection points per muscle. Head Neck 1–2/92; 14(1): 33–7.

Borodic G et al. Treatment of spasticity with botulinum toxin. Ann Neurol 1/92; 31(1): 113.

Borodic G Botulinum A toxin for (expressionisic) ptosis overcorrection after frontalis sling. Ophthal Plast Reconstr Surg 1992; 8(2): 137–42.

Borodic G et al. Botulinum A toxin for the treatment of adult–onset spasmodic torticollis. Plast Reconstr Surg 2/91; 87(2): 285–9.

(List continued on next page.)

Primary Examiner—Karen Cochrane Carlson
Assistant Examiner—Hope A. Robinson
(74) *Attorney, Agent, or Firm*—Milbank, Tweed, Hadley & McCloy LLP

(57) **ABSTRACT**

Pharmaceutical applications of a chemodenervating agent reduce pain by altering release of pain and inflammation-mediating autocoids, with a duration of action between 12–24 weeks. The limiting factor in dosing for this application is weakness and paralysis created by higher doses of the chemodenervating pharmaceutical. This weakness and paralysis is mediated by action of the neurotoxin component of the chemodenervating pharmaceutical. The invention described herein represents a novel mechanism and pharmaceutical formulation which eliminates the neurotoxin component of the chemodenervating pharmaceutical, while retaining the cytotoxin component which provides an essential bioeffect for the relief of pain and inflammation. The invention allows for improvement in administering the pharmaceutical agent for the reduction of pain and/or inflammation without causing muscular weakness and paralysis.

29 Claims, 2 Drawing Sheets

(2 of 2 Drawing Sheet(s) Filed in Color)

C. for five minutes prior to use (FIG. 1). Alternatively both stock solutions were mixed with equal volumes of 2× sample buffer without SDS and kept at −20° C. until needed (FIG. 2). Reducing agents were not used. Samples volumes were adjusted to apply 1.5 μg of protein in 2 to 5 ul volumes. SDS PAGE was conducted on 10% total acrylamide gels, with 3% cross-linking, while Native PAGE was conducted on 7.5% acrylamide gels, with 3% cross-linking. SDS-PAGE was run at 20 mA and Native PAGE was run at 10 mA, with cooling. Proteins were transferred to nitrocellulose for blotting using Tris-Glycine buffers with 20% methanol (for SDS-PAGE) or without methanol (for Native PAGE). Nitrocellulose was blocked with 5% skim milk. All primary antibodies and the secondary HRP-conjugated antibody were used at 1:2000 dilutions. The luminol-based assay system of Kirkegaard & Perry was employed to develop the blots.

Results from SDS-PAGE are shown in FIG. 1. Monoclonal antibody 7F8.G2.H3 does not detect either BNT/E_{SC} nor BNT/F when the BNT is denatured. The mouse sera from BNT/F immunized mice does recognize denatured BNT/F as well as BNT/E_{SC}. The mice had been previously immunized with BNT/E_{SC}. However mice that had been immunized only with BNT/E_{SC} had sera that recognized denatured BNT/E, but not denatured BNT/F.

Results from Native PAGE are shown in FIG. 2. Monoclonal antibody 7F8.G2.H3 does not detect native BNT/E_{SC}, but does detect native BNT/F. The mouse sera from BNT/F immunized mice still recognize BNT/F as well as BNT/E_{SC}. Mice that had been immunized with BNT/E_{SC} have sera that recognize BNT/E, but also recognize native BNT/F.

In Vivo Neutralization Assay

Mice received an intravenous (IV) injection of 50 μl of the stock ascites antibody (7F8.G2.H3) in PBS (280 ug mouse antibody per mouse) or PBS. One hour later they were challenged with 5 IP mLD50 (280 pg/mouse). Results are expressed as survivors/total.

In vivo neutralization assay:

Group	Day	1 S/T	2 S/T	3 S/T	6 S/T	10 S/T	21 S/T
7F8 Ascites		4/4	4/4	4/4	4/4	4/4	4/4
PBS		1/4	0/4				

Second In Vivo Neutralization Assay

Mice received an intravenous (IV) injection of 50 μl of the stock ascites antibody (7F8.G2.H3) in PBS (280 ug mouse antibody per mouse) or PBS. One hour later they were challenged with varying IP mLD_{50} doses. Results are expressed as survivors total.

DATA is expresses as Survivors/Total

Group IV		BNT/F IP mLD_{50}	1d	2d	3d	5d	21d
Control 1	PBS	5	2/3	2/31	1/3	1/3	1/3
Control 2	PBS	50	0/3				
Test 1	7F8	5	4/4	4/4	4/4	4/4	4/4
Test 2	7F8	50	4/4	4/4	4/4	4/4	4/4
Test 3	7F8	500	4/4	1/4	1/4	1/4	1/4
Test 4	7F8	5000	1/4	1/4	1/4	1/4	1/4
Test 5	7F8	50000	0/4				

What is claimed is:

1. A neutralizing monoclonal antibody specific to the active form of botulinum neurotoxin serotype F, BNT/F.

2. A monoclonal antibody according to claim 1 which is produced by a hybridoma between a mouse myeloma cell and a spleen cell of a mouse immunized with BNT/F.

3. A monoclonal antibody according to claim 2 wherein said hybridoma is F197/7F8.G2.H3 having ATCC designation HB-12102.

4. A monoclonal antibody according to claim 3 wherein said monoclonal antibody is 7F8.G2.H3.

5. A hybridoma having ATCC designation HB12102 which produces a monoclonal antibody according to claim 1 and progeny of said hybridoma.

6. A protein comprising the antigen binding domain of the monoclonal antibody of claim 4.

7. A neutralizing monoclonal antibody according to claim 1 produced by the method comprising the step of immunizing with native botulinum neurotoxin serotype F.

* * * * *

US005807741A

United States Patent [19]

Brown et al.

[11] Patent Number: 5,807,741

[45] Date of Patent: Sep. 15, 1998

[54] NEUTRALIZING MONOCLONAL ANTIBODY AGAINST BOTULINUM NEUROTOXIN SEROTYPE F

[76] Inventors: **Douglas Richard Brown**, 8917 N. Westland, Gaithersburg, Md. 20877; **James Jude Schmidt**, 5819 Catoctia Vists, Mount Airy, Md. 21771

[21] Appl. No.: **504,969**

[22] Filed: **Jul. 20, 1995**

[51] **Int. Cl.**[6] **C12N 5/12**; A61K 39/395

[52] **U.S. Cl.** **435/340**; 435/70.21; 435/172.2; 530/388.4; 530/387.3; 424/164.1; 424/247.1

[58] **Field of Search** 424/150.1, 164.1, 424/163.1, 247.1; 435/69.6, 70.21, 172.27, 240.27, 340; 530/388.4, 389.5, 387.3

[56] **References Cited**

PUBLICATIONS

Paul, WE. 1993 Fundamental Immunology p.242.

Primary Examiner—Lila Feisee
Assistant Examiner—Julie E. Reeves
Attorney, Agent, or Firm—John F. Moran; Sana A. Pratt

[57] **ABSTRACT**

Antibodies which neutralize botulinum neurotoxin serotype F are produced using biologically active botulinum neurotoxin instead of toxoid for immunization and exploiting the importance of cross reaction between various serotypes to obtain immune responses, or monoclonal antibodies, to additional serotypes of interest. Methods of preparation and uses of the neutralizing botulinum neurotoxin antibodies are described.

7 Claims, 2 Drawing Sheets

DERWENT-ACC-NO: 2004-652009
DERWENT-WEEK: 200560

TITLE: New isolated antibody that neutralizes botulinum neurotoxin type A, useful for diagnosing botulism or for treating pathologies associated with botulinum neurotoxin poisoning

Basic Abstract Text (1):
NOVELTY - An isolated antibody (I) that specifically binds to an epitope specifically bound by an antibody expressed by a specific clone where (I) binds to and neutralizes botulinum neurotoxin type A (BoNT/A), is new.

Basic Abstract Text (4):
(1) a polypeptide (II) comprising BoNT/A neutralizing epitope having an epitope that is specifically bound by an antibody expressed by clones as mentioned in (I);

Basic Abstract Text (6):
(3) a composition (III) comprising several anti-botulinum neurotoxin antibodies, where each antibody is specific for a different epitope of a botulinum neurotoxin and the combination of antibodies shows greater toxin neutralization than the single antibodies in surplus.

Basic Abstract Text (9):
USE - (I) is useful for neutralizing BoNT/A antibody which involves contacting BoNT/A with (I) (first antibody) having specificity and affinity such that it specifically binds to and neutralizes BoNT/A. The above method further involves contacting BoNT/A with a second type of (I) that has specificity and affinity such that it specifically binds to and neutralizes BoNT/A, where the second antibody binds to a different epitope than the first antibody. (I) is useful for neutralizing a botulinum neurotoxin which involves contacting neurotoxin with (I) in surplus, where each of (I) is specific for a different epitope of the botulinum neurotoxin and the combination of antibodies shows greater toxin neutralization than the single antibodies in surplus (all claimed). (I) is useful for diagnosing the botulism or for treating pathologies associated with botulinum neurotoxin poisoning.

-continued

```
Lys Leu Val Ala Ser Asn Trp Tyr Asn Arg
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Gln Ile Glu Arg Ser Ser Arg Thr Leu Gly
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Cys Ser Trp Glu Phe Ile Pro Val Asp Asp
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Gly


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                15                  20

Asp Asn Phe Thr Asn
                25
```

What is claimed is:

1. Monoclonal antibody 6B2-2, produced from hybridoma cell line having accession number ATCC PTA-969.

2. A composition comprising the monoclonal antibody of claim 1.

3. The continuous hybridoma cell line having deposit accession number ATCC PTA-969, and clones thereof.

4. A method for detecting BoNT/A said method comprising:

(i) incubating a sample with an effective amount of one or more monoclonal antibodies with specificity for BoNT/A, said monoclonal antibodies comprising 6B2-2 produced by the hybridoma cell line having the accession number ATCC PTA-969, under conditions which allow the formation of an antibody-BoNT/A complex; and

(ii) detecting the antibody-BoNT/A complex wherein the presence or absence of the complex correlates to the presence or absence of BoNT/A in the sample.

5. The method for detecting BoNT/A according to claim 4 wherein, said sample is water, biological, pharmaceutical, or food products.

6. A kit for detecting BoNT/A in a biological sample, said kit comprising:

(1) a container comprising monoclonal antibody 6B2-2 produced by the hybridoma cell line having the accession number ATCC PTA-969; and

(2) instructions for using the antibody for the purpose of binding to BoNT/A to form an immunological complex and detecting said immunological complex such that the presence or absence of said immunological complex correlates to the presence or absence of BoNT/A in said sample.

7. A method for capturing BoNT/A from a sample, said method comprising contacting said sample with the monoclonal antibody 6B2-2 produced by the hybridoma cell line having the accession number ATCC PTA-969, and isolating the complex formed between the BoNT/A in the sample and the monoclonal antibody.

8. The method according to claim 7 wherein said sample is selected from the group consisting of: biological fluid and animal tissue.

* * * * *

US006667158B1

(12) United States Patent
Bavari et al.

(10) Patent No.: US 6,667,158 B1
(45) Date of Patent: Dec. 23, 2003

(54) ANTIBODIES AGAINST TYPE A BOTULINUM NEUROTOXIN

(75) Inventors: Sina Bavari, Frederick, MD (US); Edna R. Torres Melendez, Frederick, MD (US); Frank J. Lebeda, Phurmont, MD (US)

(73) Assignee: The United States of America as represented by the Secretary of the Army, Washington, DC (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: 09/465,276

(22) Filed: Dec. 16, 1999

Related U.S. Application Data

(60) Provisional application No. 60/112,632, filed on Dec. 17, 1998.

(51) Int. Cl.[7] G01N 33/569

(52) U.S. Cl. 435/7.32; 435/7.1; 530/388.1; 530/388.2; 530/388.4; 424/164.1

(58) Field of Search 530/350, 388.1, 530/388.2, 388.4; 424/164.1, 167.1; 435/7.1, 7.32

(56) References Cited

U.S. PATENT DOCUMENTS

6,287,566 B1 * 9/2001 Dertzbaugh
2002/0034521 A1 * 3/2002 Lee et al.

OTHER PUBLICATIONS

Bavari et al. Vaccine, Nov. 1998, vol. 16 No. 19 pages 1850–1856.*

* cited by examiner

Primary Examiner—Lynette R. F. Smith
Assistant Examiner—Robert A. Zeman
(74) *Attorney, Agent, or Firm*—Elizabeth Arwine; Charles H. Harris

(57) ABSTRACT

Antibodies for binding epitopes of BoNT/A and hybridomas which produce such antibodies are described. The antibodies of the present invention can be used in a method for detecting BoNT/A in a sample and/or in a method for purifying BoNT/A from an impure solution. In addition, the antibodies can be used for passive immunization against BoNT/A intoxication or as intoxication therapy. Another aspect of the invention is a kit for detecting BoNT/A in a sample.

8 Claims, 15 Drawing Sheets

L5: Entry 40 of 42 File: DWPI Sep 15, 2005

DERWENT-ACC-NO: 2002-479492
DERWENT-WEEK: 200569

TITLE: Detecting Clostridium botulinum neurotoxin serotypes A, B, E and F, using a sensitive and specific enzyme linked immunosorbant assay

Basic Abstract Text (1):
NOVELTY - An assay for detecting botulinum neurotoxins comprising using antibodies which are affinity-purified to the heavy chain C fragment of the toxin, so that the antibodies do not cross-react between serotypes, recognize neutralizing epitopes, and recognize purified and complexed toxins equally, is new.

Equivalent Abstract Text (1):
NOVELTY - An assay for detecting botulinum neurotoxins comprising using antibodies which are affinity-purified to the heavy chain C fragment of the toxin, so that the antibodies do not cross-react between serotypes, recognize neutralizing epitopes, and recognize purified and complexed toxins equally, is new.

US 20020058294A1

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2002/0058294 A1

Poli et al. (43) Pub. Date: May 16, 2002

(54) METHOD FOR DETECTING CLOSTRIDIUM BOTULINUM NEUROTOXIN SEROTYPES A, B, E AND F IN A SAMPLE

(76) Inventors: Mark A. Poli, Frederick, MD (US); Victor R. Rivers, Frederick, MD (US)

Correspondence Address:
U.S. Army Medical Research and Materiel Command
ATTN: MCMR-JA (Ms. Elizabeth Arwine-PATENT ATTY)
504 Scott Street
Fort Detrick, MD 21702-5012 (US)

(21) Appl. No.: 09/952,078

(22) Filed: Sep. 14, 2001

Related U.S. Application Data

(63) Non-provisional of provisional application No. 60/232,929, filed on Sep. 15, 2000.

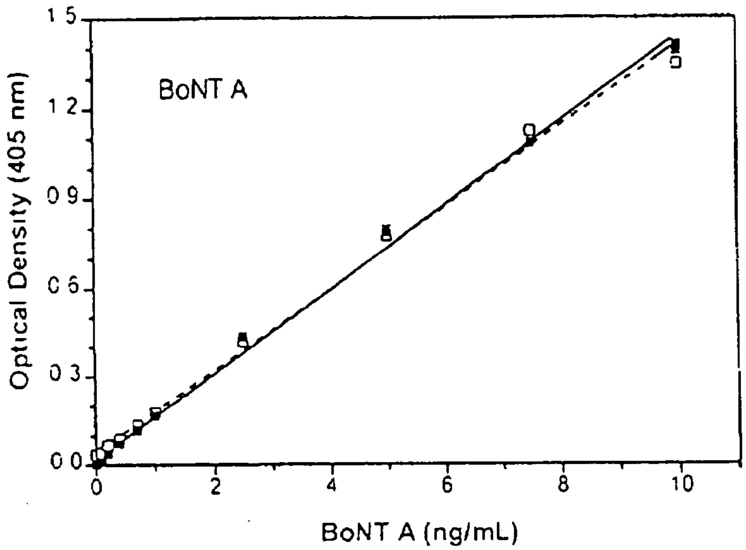
Publication Classification

(51) Int. Cl.[7] G01N 33/554; G01N 33/569; G01N 33/53

(52) U.S. Cl. 435/7.32; 435/7.5

(57) ABSTRACT

Sensitive and specific enzyme-linked immunosorbent assays which detect Clostridium botulinum neurotoxins serotypes A, B, E, and F in a sample are described. The assay is based upon affinity-purified antibodies directed against the C-fragments of each toxin. These assays demonstrate sensitivity close to that of the mouse bioassay without the use of animals and in a much simpler format than other assays of similar sensitivity.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BoNT A (ng/mL)
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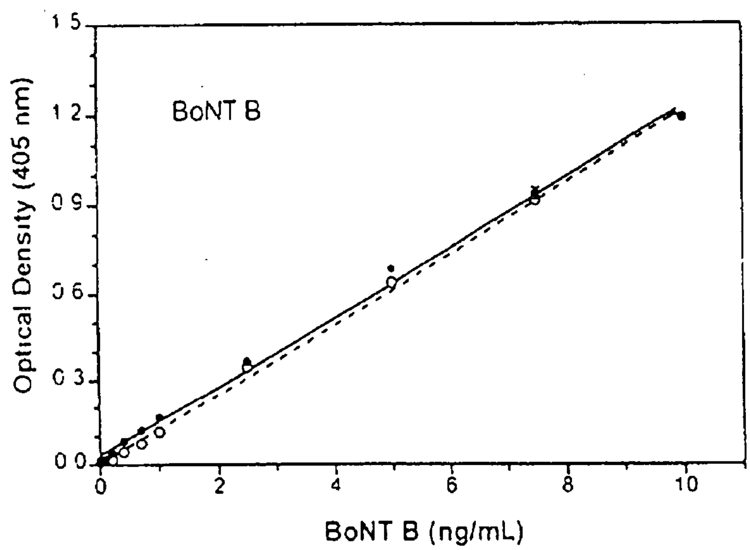
6
8
10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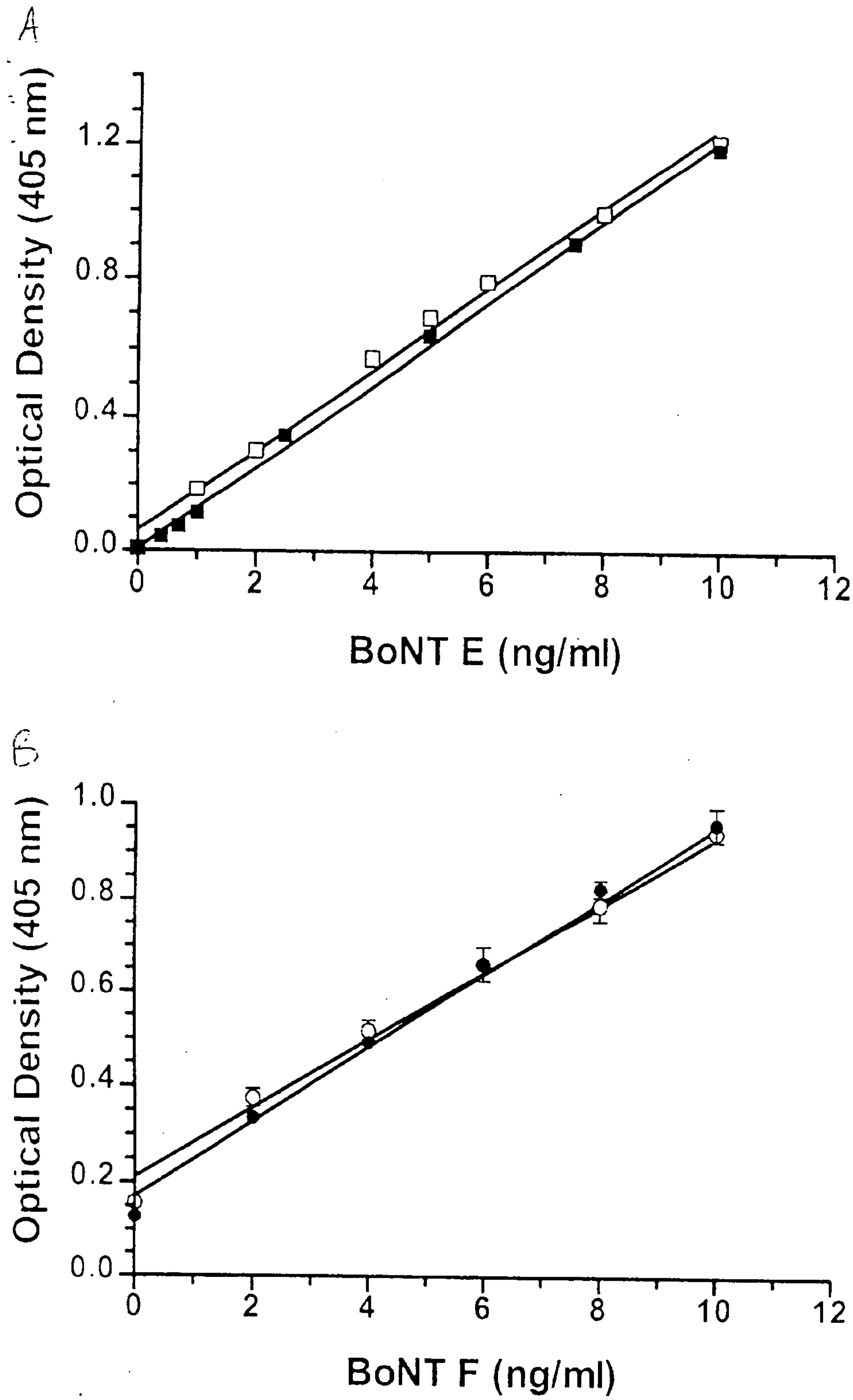
8
10

FIGURE 1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0.0
0
2
4
6
8
10
12
BoNT E (ng/ml)
B
Optical Density (405 nm)
1.0
0.8
0.6
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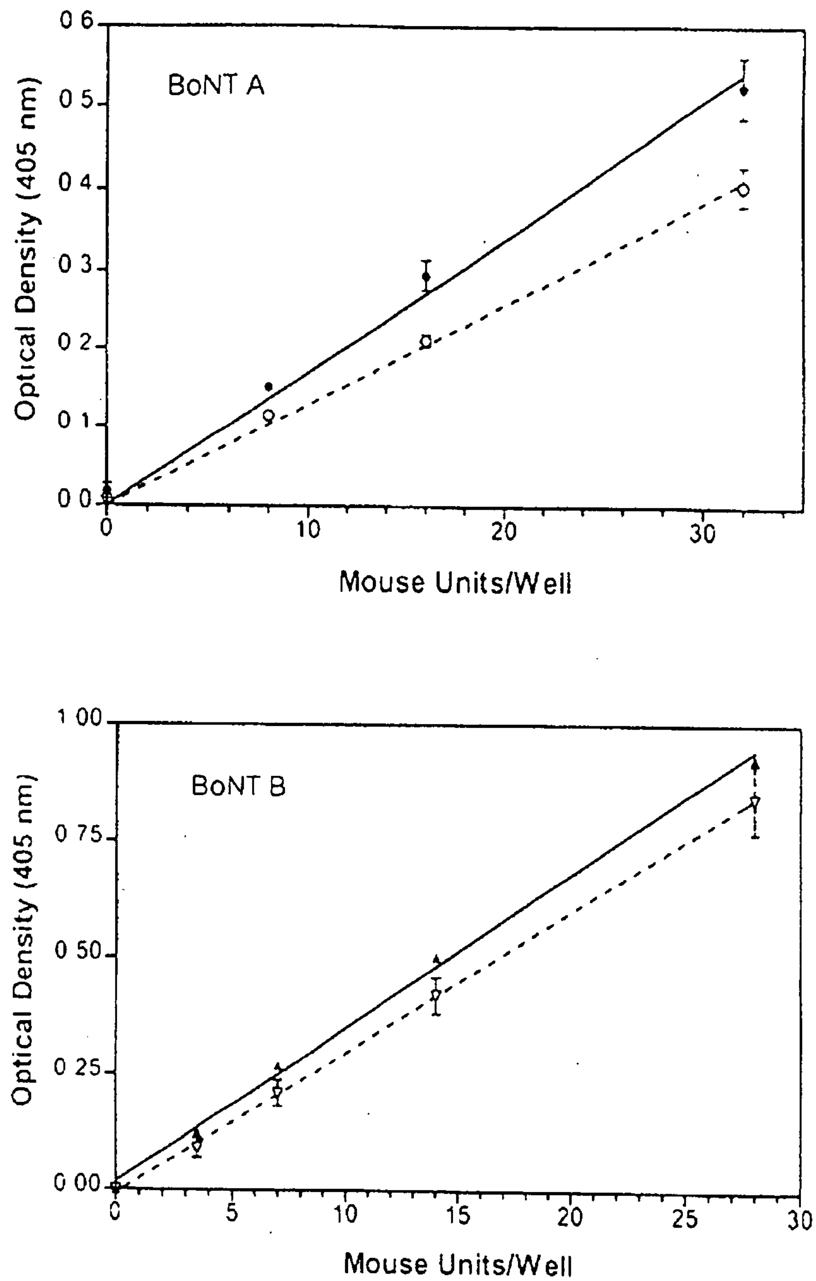
0.2
0.0
0
2
4
6
8
10
12
BoNT F (ng/ml)

FIGURE 2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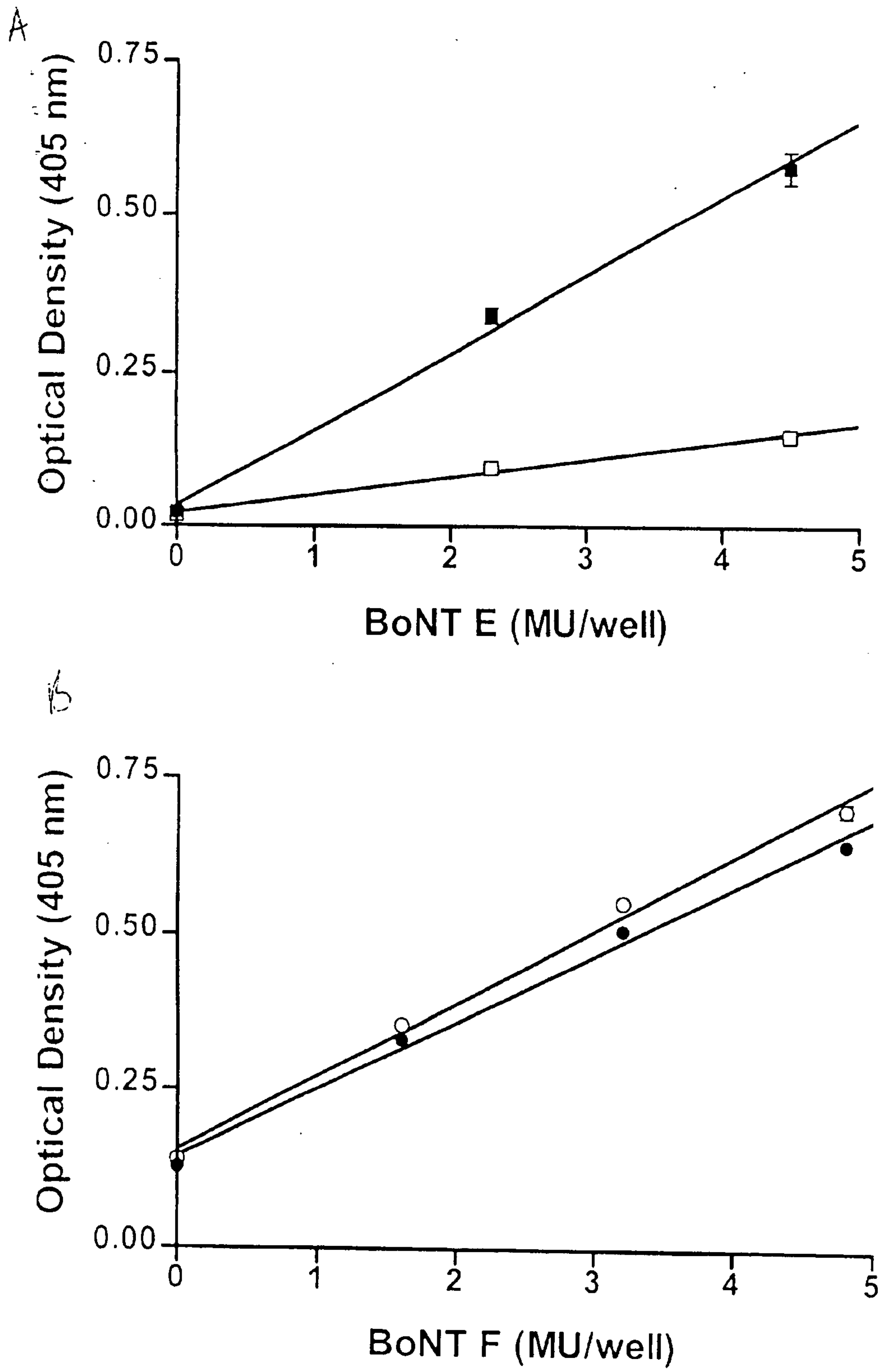
BoNT B
Optical Density (405 nm)
Mouse Units/Well

FIGURE 3


A
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0.00
Optical Density (405 nm)
0
1
2
3
4
5
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B
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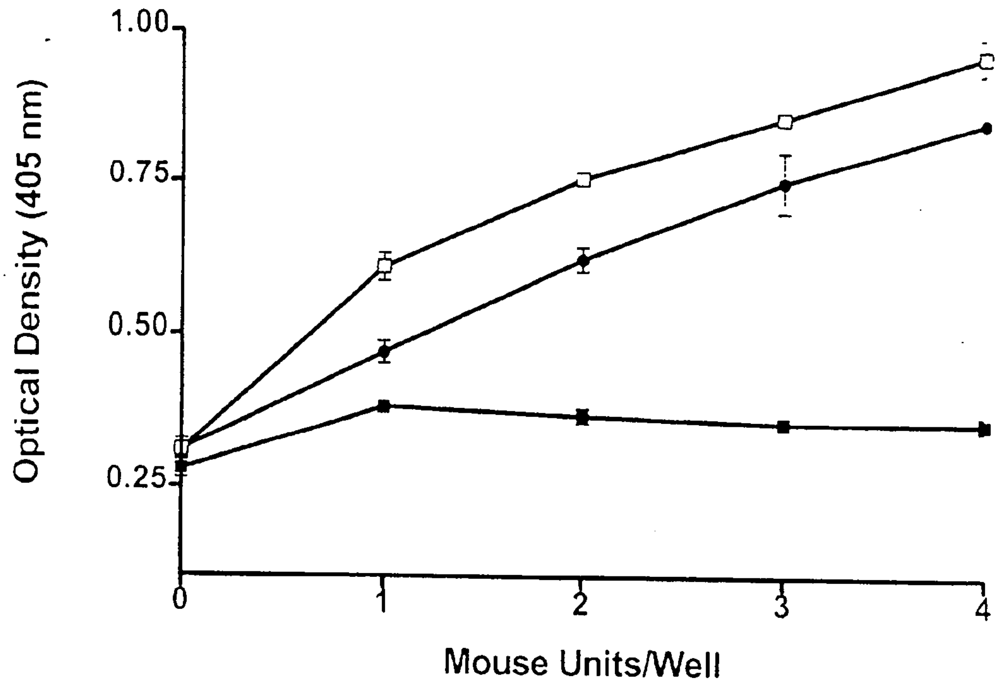
4
5
BoNT F (MU/well)

FIGURE 4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2
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Mouse Units/Well

FIGURE 5

METHOD FOR DETECTING CLOSTRIDIUM BOTULINUM NEUROTOXIN SEROTYPES A, B, E AND F IN A SAMPLE

BACKGROUND OF THE INVENTION

[0001] The present invention relates to a sensitive and specific enzyme-linked immunosorbent assay for the detection of Clostridium botulinum neurotoxin serotypes A (BoNT A), B (BoNT B), E (BoNT E) and F (BoNT F) in a sample.

[0002] The anaerobic bacterium *Clostridium botulinum* produces seven serologically different toxins. Recognized as the most potent toxins of biological origin, botulinum neurtoxins (BoNTs) are the causative agents of food-borne, infant and wound botulism (Sakaguchi, 1983, Pharmac. Ther. 19, 165-194). The toxins act presynaptically at the neural junction by blocking the release of acetylcholine and thereby causing a flaccid muscular paralysis (Simpson, 1986, Ann. Rev. Pharmacol. Toxicol. 26, 427-453). Paralysis of the respiratory musculature can cause death in untreated patients. All serotypes (MW approximately 150 kDa) consist of two polypeptide subunits joined by an intra chain disulfid bridge and are bound to nontoxic neurotoxin-associated proteins (NAP's) which protect the toxins in the gastrointestinal tract. The mechanism of action is similar for each serotype. The heavy chain (B chain) is the binding subunit, which binds to a receptor on the presynaptic membrane. The light chain (A chain) is the catalytic subunit. Once translocated across the cell membrane, its zinc-dependent protease activity hydrolyzes specific proteins associated with synaptic vesicle docking and acetylcholine release (Schiavo et al., 1994, Sem. Cell. Biol. 5, 221-229).

[0003] Currently, the mouse bioassay is the most widely accepted method for detecting BoNT's in serum and foods. This assay has the desired sensitivity (<5 mouse lethal units/mL), but it is cumbersome, time consuming (1-4 days) and involves the use of large numbers of animals (Shone et al., 1985, Appl. Environ. Microbiol. 50, 637-667). Enzyme-linked immunosorbent assays (ELISA's) have been reported by several laboratories (Dezfulian and Bartlett, 1984, J. Clin. Microbiol. 19, 645-648; Shone et al., 1985, supra), but lack the required sensitivit. An enzyme-linked coagulation assay (ELCA) was reported with a sensitivity comparable to the mouse bioassay (Doellgast et al., 1994, J. Clin. Microbiol. 32, 851-853), but this assay relies upon a sophisticated amplification system utilizing a snake venom coagulation factor and is limited by it's complexity and the expense of the reagents.

[0004] Therefore, there is a need for a simple, rapid sensitive, and accurate assay for the measurement of BoNT's in samples without the use of animals or complicated and expensive reagents.

SUMMARY OF THE INVENTION

[0005] The present invention satisfies the need discussed above. The present invention relates to a simple, sensitive colorimetric capture ELISA for BoNTs with detection limits at or below 1 mouse unit. The assay is reproducible and accurate with negligible cross-reactivity between serotypes. The strength of the assay relies on its novel format and the unique preparation of the antibodies used in the assay. The antibodies are affinity-purified to the heavy chain C-fragment of the toxin. Others have used antibodies which are not affinity purified or which are purified to the whole toxin molecule. We reasoned that since the C-terminal region of the heavy chain is where the binding domain is located, this portion of the molecule should not be covered by associated proteins; if the binding domain was blocked, then the molecule would be precluded from binding to the cell surface and would not be toxic. Thus, the binding region "looks" the same in both the purified and complexed forms. Antibodies to this region should recognize both forms of the toxin. The result of the unique preparation of the antibodies is that they do not cross-react between serotypes, they recognize neutralizing epitopes, and they recognize purified and complexed toxins equally.

BRIEF DESCRIPTION OF THE DRAWINGS

[0006] These and other features, aspects, and advantages of the present invention will become better understood with reference to the following description and appended claims, and accompanying drawings where:

[0007] **FIGS. 1A and 1B**. Colorimetric ELISAs for BoNT serotypes A and B. Assays were performed as described in Materials and methods in assay buffer (open symbols) or 10% human serum (solid symbols). Points are means of triplicate determinations. Where not visible, error bars are within the symbol.

[0008] **FIGS. 2A and 2B**. Colorimetric ELISAs for BoNT serotypes E and F. Assays were performed as described in Materials and Methods in assay buffer (open symbols) or 10% human serum (solid symbols). Points represent means of triplicate determinations. Where not visible, error bars are within the symbol.

[0009] **FIGS. 3A and 3B**. Comparison of ELISA detection of purified neurotoxin (solid symbols) and toxin complexed with associated nontoxic proteins (open symbols) for BoNT A and B. Assays were performed as described in Materials and Methods. Points are means of triplicate determinations. Where not visible, error bars are within the symbols.

[0010] **FIGS. 4A and 4B**. Comparison of ELISA detection of purified neurotoxin BoNT E and F (open symbols) and toxin complexed with associated nontoxic proteins (solid symbols). Assays were performed as described in Materials and Methods. Points represent means of triplicate determinations. Where not visible, error bars are within the symbols.

[0011] **FIG. 5**. Comparison of trypsinized and non-trypsinized BoNT E. Purified BoNT E, either trypsinized (solid squares) or non-trypsinized (open squares), and trypsinized BoNT E complex (solid circles) were analyzed by ELISA according to Materials and Methods. Trypsinized (activated) complex and trypsinized purified toxin were used at equal mouse unit concentrations; non-trypsinized BoNT E was used at used at equal mass to trypsinized BoNT E. Trypsinizing purified toxin destroys antigenic determinants, while NAPs significantly protect the complexed toxin. Points represent means of triplicate determinations.

DETAILED DESCRIPTION OF THE INVENTION

[0012] A capture ELISA method comprises the use of two (monoclonal or polyclonal) antibodies to the same antigen

with two different epitopes, one of which is conjugated with biotin. The antigen containing supernatant is reacted with the first antibody and washed with a buffer solution. The antibody linked antigen is then reacted with the second biotinylated antibody and then washed to remove the excess antibody. The antibody-antigen-antibody/biotin is then cross-linked with avidin optionally linked to a chromogenic enzyme and washed. Finally, a substrate is reacted with the avidin or the chromogenic enzyme and the development of color product is measured.

[0013] In one embodiment, the present invention provides a method for detecting BoNT comprising:

[0014] (a) reacting a sample suspected of having an antigen with a first antibody, Ab(A);

[0015] (b) washing the reaction mixture to remove unreacted antibody;

[0016] (c) reacting the Ab(A) linked antigen with a second antibody which is conjugated with biotin, Ab(B);

[0017] (d) washing the reaction mixture to remove unreacted antibody;

[0018] (e) reacting Ab(A)-antigen-Ab(B) with avidin linked to a chromogenic enzyme;

[0019] (f) washing the reaction mixture;

[0020] (g) reacting the chromogenic enzyme;

[0021] (h) measuring optical density of reaction and calculating concentration of antigen using a standard curve.

[0022] A sample includes any solution which is suspected of containing BoNT such as animal serum including human serum, food samples, and dirt samples.

[0023] The antibody against BoNT can be of any isotype, such as IgA, IgG or IgM, Fab fragments, or the like. The antibody may be a monoclonal or polyclonal and produced by methods as generaly described in Harlow and Lane, *Antibodies, A Laboratory Manual,* Cold Spring Harbor Laboratory, 1988, incorporated herein by reference. Any monoclonal which has the desired specificity and affinity can potentially be used once the conditions for use of the antibody are tested. Preferably, polyclonal antibodies are used since polyclonal antibodies can recognize different epitopes of BoNT protein thereby enhancing the sensitivity of the assay. Polyclonal antibodies for use in the assay can be any polyclonal available commercially with the requisite affinity and specificity. It is preferable that the antibodies are affinity purified to the C-fragment of BoNT as described in the examples below so that the antibody may recognize purified and complexed toxin equally. The antibodies used to measure BoNTs in the examples below were serotype specific polyclonal antibodies purified from hyperimmune horse serum. Any serum from any other source could potentially work as long as the affinity of the antibody to the antigen is high enough to result in detection, i.e. the detection limit of the assay will be dictated by the affinity of the antibody. Most preferably, the antibody is affinity-purified against the C-fragment of BoNT. Antibodies were affinity purified over a column with heavy-chain C-fragment of specific serotypes to produce Ab(A). The affinity purified antibodies were labeled with biotin to produce biotinylated antibody Ab(B). Any other label can be used which provides high affinity between the label and the detection molecule. Care must be taken that the coupling of the BoNT to the label will not inactivate the enzyme. Also, it is important that the background be low since high background would adversly effect the detection limit.

[0024] The antibody can be applied to the solid support by direct or indirect means. Indirect bonding allows maximum exposure of the toxin binding sites to the assay solutions since the sites are not themselves used for binding to the support. The solid support can be a any phase used in performing immunoassays, including dipsticks, membranes, absorptive pads, beads, microtiter wells, test tubes, and the like. Preferred are microtiter plates which produce low background noise and can bind a high amount of capture antibody in the wells. These factors affect signal-to-noise ratios and hence assay performance. In addition, most plate readers are set up for 96-well plates as described in Materials and Methods below but other plates can be used if a washer/reader is adjusted or designed for it. If a plate is used, the anti-BoNT antibody is bound to the plate using directions from the manufacturer or any other method known to the investigator.

[0025] The solid support is preferably non-specifically blocked after binding the BoNT antibodies to the solid support. Non-specific blocking of surrounding areas can be with Superblock™ blocking reagent from Pierce (Rockford, Ill.), whole or derivatized bovine serum albumin, or albumin from other animals, whole animal serum, casein, non-fat milk, and the like.

[0026] The sample is applied onto the solid support with bound BoNT-specific antibody such that the BoNT protein will be bound to the solid support through said antibodies. Excess and unbound components of the sample are removed and the solid support is preferably washed so the antibody-antigen complexes are retained on the solid support. The solid support may be washed with a washing solution which may contain a detergent such as Tween-20, Tween-80, or any other washing solution as long as it does not denature proteins and inferfere with protein/protein interactions.

[0027] After the BoNT has been allowed to bind to the solid support, a second antibody which reacts with BoNT is applied. The second antibody may be labeled, preferably with a visible label. The labels may be soluble or particulate and may include dyed immunoglobulin binding substances, simple dyes or dye polymers, dyed latex beads, dye-containing liposomes, dyed cells or organisms, or metallic, organic, inorganic, or dye solids. The labels may be bound to the BoNT antibodies by a variety of means that are well known in the art. In some embodiments of the present invention, the lables may be enzymes that can be coupled to a signal producing system. Examples of visible labels include alkaline phosphatase, beta-galactosidase, horseradish peroxidase, and biotin. The steptavidin-phosphatase could be substituted by any streptavidin or avidin-linked indicator system, for example streptavidin-peroxide, Europium, or chemilunescence and its respective developing substrate or indicator, generically referred to as substrate. Many enzyme-chromogen or enzyme-substrate-chromogen combinations are known and used for enzyme-linked assays.

[0028] Preferably, the affinity purified BoNT antibody is used as a second antibody to bind the BoNT-antibody Ab(A)

complex. The second antibody is preferably labeled with biotin producing a biotinylated BoNT antibody, Ab(B). After binding Ab(B) to the Ab(A)-BoNT complex, the plates are washed and neutravidin-linked alkaline phosphatase is added. After binding of the neutravidin to the Ab(B)-BoNT-Ab(A) complex, enzyme substrate p-nitrophenyl phosphate was added and the color allowed to develop.

[0029] Accumulated label or color may be detected by optical detection devices such as reflectance analyzers, video image analyzers and the like. The visible intensity of accumulated label could correlate with the concentration of BoNT in the sample. The correlation between the visible intensity of accumulated label and the amount of BoNT may be made by comparison of the visible intensity to a set of reference standards. Preferably, the standards have been assayed in the same way as the unknown sample, and more preferably alongside the sample, either on the same or on a different solid support. The concentration of standards to be used can range from about 0.1 ng of BoNT per ml of solution, up to about 10 mg of BoNT per ml of solution Toxicon 34(9), 975-985). Preferably, several different concentrations of BoNT are used so that quantitating the unknown by comparison of intensity of color is more accurate. Additionally, BoNT complexed with nontoxic-neurotoxin associated proteins can be used to establish a standard curve. If one is trying to detect complexed toxin, one should use complexed toxin in the standard curve. This gives the best accuracy. Naturally-occurring BoNT intoxications are all caused by complexed toxin. The fact that this assay recongizes both forms the free and complexed forms of the toxin is one of its strengths.

[0030] As evident to a person with ordinary skill in the art, it may be necessary to undergo one or more serial dilutions of the sample such that the level of BoNT in the sample can be compared to one of the set standards. The BoNT measurement is then corrected for the dilution factor.

[0031] All the materials and reagents required for assaying BoNT according to the present invention can be assembled together in a kit. This generally will comprise one or more solutions containing a known concentration of BoNT, a washing solution, a solution of a chromogen which changes color or shade by the action of the enzyme directly or indirectly through action on a substrate, a anti-BoNT conjugated to a label such that it could be detected and an anti-BoNT antibody without a label, pipettes for the transfer of said solutions, test tubes for said solutions, and a solid support carrying on the surface thereof a polyclonal antibody to BoNT. The kit may also contain one or more solid support having an anti-BoNT antibody for use in assaying one or more samples simultaneously or individually, and the necessary reagent required to develop the label. It is also preferable that the BoNT used for standards, whether free or complexed with a substrayte, be provided so that it could be assayed fresh along with the unknown sample. Such kits will comprise distinct containers for each individual reagent. The reagents may be supplied from storage bottles or one or more of the test tubes may be prefilled with the reagents or controls.

[0032] The components of the kit may also be provided in dried or lyophilized forms. When reagents or components are provided as a dried from, reconstitution generally is by the addition of a suitable solvent. It is envisioned that the solvent also may be provided in another container means.

[0033] In any of the above test kits, all solutions and antibodies for detection of one BoNT can be provided in one kit. Alternatively, solutions and antibodies for more than one serotype can be provided in one kit. In addition, a test plate can be prepared such that different parts of the plate can be used to detect a different serotype of BoNT.

[0034] The kits of the present invention also will typically include a means for containing the reagents such as vials or tubes in close confinement for commercial sale such as, e.g. injection or blow-molded plastic containers into which the desired vials are retained.

[0035] The following examples are included to demonstrate an embodiment of the invention. It should be appreciated by those of skill in the art that in light of the present disclosure, many changes can be made in the specific embodiment disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

[0036] The following MATERIALS AND METHODS were used in the examples that follow.

[0037] Toxins

[0038] Purified *Clostridium botulinum* neurotoxin serotypes A and B (1.6×10^8 and 7.0×10^7 mouse i.p. LD_{50}'s/mg, respectively) and neurotoxins A and B complexed with NAPs (4.5×10^7 and 1.4×10^7 mouse i.p. LD_{50}'s/mg, respectively) were purchased from the University of Wisconsin Food Research Institute (Madison, Wis.). Purified *C. botulinum* neurotoxin serotypes E and F (4.5×10^7 and 8.0×10^6 mouse i.p. LD_5's/mg, respectively) and neurotoxins E and F complexed with NAPs (2×10^6 and 4×10^6) mouse i.p. LD_{50}'s/mg, respectively) were purchased from METAbiologics, Inc, Madison, Wis. Stock solutions (10 μg/mL), were kept at 40° C. in sterile buffer [50 mM sodium acetate pH 4.2, 2% gelatin, and 3% bovine serum albumin (BSA)]. Working dilutions were prepared immediately before use. Heavy-chain C-fragments were purchased from Ophidian Pharmaceuticals (Madison, Wis.) and stored at 40° C. before use.

[0039] Preparation of affinity columns

[0040] Cyanogen bromide-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) was hydrated in 1 mM HCl for 4 hr at room temperature with gentle stirring, and then washed with coupling buffer (0.1 M $NaHCO_3$, 0.5 M NaCl, pH 8.3). Heavy-chain C-fragment (10 mg) dissolved in 5 mL Coupling buffer was added to 3 g of washed beads and slowly rotated for 5 hr at room temperature. After centrifugation for 15 min at 3,000×g, the pellet was resuspended in 15 mL of blocking buffer (0.1 M $NaHCO_3$, 0.2 M glycine, pH 8.0) and gently rotated overnight at 40° C. The coupled gel was then poured into a column and the beads were allowed to settle. After washing with three column volumes of wash buffer (0.1 M $NaC_2H_3O_2$, 0.5 M NaCl, pH 4.0) followed by three column volumes of coupling buffer, the column was equilibrated in phosphate-buffered saline (PBS) containing 0.9% benzoyl alcohol as a bacteriostatic agent and refrigerated.

[0041] Hyperimmunization of horses: Animals were immunized with at least three doses of monovalent botulinum toxoid, one serotype (A,B,C,D,E,F, or G) per horse, and subsequently boosted with botulinum toxin of the same serotype. Alum precipitated toxoid in Ribi adjuvant or toxin in saline was administered subcutaneously in multiple sites

in the neck and flank to increase absorption of antigenic materials, and to minimize the potential for focal inflammation (18-21 ga needle). Only the first injection of toxoid was administered with an adjuvant. Subsequent boosters consisted of toxoid or botulinum toxin only. Injections of toxoid or, toxin were given within six inches of a potential site of blood withdrawl. Horses were confined to the USAMRIID Large Animal Research Facility (LARF) barn or an immediately adjacent paddock for at least one hour after administration of toxoid or toxin for observation for adverse reactions. An anaphylaxis kit was immediately available and horses were monitored during the observation period. When the animals are plasmapheresed, they were kept in the LARF barn overnight to facilitate observation during the post bleeding period.

[0042] The immunization schedule was one of the following for each of the animals:

[0043] Immunization Schedule (first six horses)

[0044] Week 0: 2.0 mg of toxoided neurotoxin *—and one dose of tetanus toxoid (three horses will receive Complete Freunds Adjuvant (CFA) and three additional horses receiving Ribi adjuvant with botulinum toxoid—see adjuvant, IV.C.5. Only the primary injection will contain an adjuvant)

[0045] Day 4: Bleed for lymphocyte stimulation assay (10 ml)

[0046] Week 2: 0.5 mg of toxoided neurotoxin **

[0047] Week 4: Bleed for titer (10 ml)

[0048] Week 5: 0.5 mg of toxioded neurotoxin **

[0049] Week 5+3, 6, & 9 days: Bleed for titer & IgG/IgM ratio (10 ml)

[0050] Week 5+12 days: Bleed for IgG/IgM ratio and titer

[0051] Week 5+16 days: Begin plasmapheresis every 3 days for 12 bleeds

[0052] (8-12 liters per bleed).

[0053] After 12 plasmaphereses, rest for 1 week and boost, entering the schedule again at week 5 (52 day cycle).

[0054] Immunization Schedule: (sixty nine horses)

[0055] Week 0: 2.0 mg of toxoided neurotoxin *

[0056] Week 2: 0.5 mg of toxoided neurotoxin **

[0057] Week 4: Bleed for titer (10 ml)

[0058] Week 5: 0.5 mg of toxioded neurotoxin **

[0059] Week 5+12 days: Bleed for IgG/IgM ratio titer

[0060] Week 5+16 days: Begin plasmapheresis every 3 days for 12 bleeds

[0061] (8-12 liters per bleed)

[0062] After 12 plasmaphereses, rest for 1 week and boost (52 day cycle)

*Crude toxoid is formalin toxoided neurotoxin of 10-20% purity

**Pure toxoid is formalin toxoided neurotoxin of 85-95% purity

[0063] Animals were bled (10 ml) and their plasma examined for anti-botulinum antibody with a mouse neutralization assay 12 days after the second immunization of the initial series. Horses that had not mounted an adequate antibody response were evaluated and a determination was made as to whether to continue or to remove the horse from the protocol. As serological results from early groups become available, schedules were modified for remaining animals.

[0064] Affinity purification of Anti-BoNT antibodies

[0065] Antibodies against specific serotypes were purified from hyperimmune horse serum. The serum was first precipitated by gradually adding an equal volume of 90%-saturated ammonium sulfate followed by gentle stirring for 2 hr at room temperature. The solution was then sedimented at 3000×g for 15 min. The pellet was taken up in the original volume of PBS, and then dialyzed against three to five changes of PBS to remove residual ammonium sulfate. This crude antibody fraction (50 mL) was circulated twice over the appropriate C-fragment affinity column. The column was then washed with three column volumes of PBS and the BoNT-specific antibody eluted with 0.1 M glycine, pH 3.0, and immediately neutralized with 0.1 volumes of 10×PBS. The eluted fraction was dialyzed extensively against distilled water, lyophilized and stored at 40° C.

[0066] Biotinylation of affinity-purified antibody

[0067] Affinity-purified antibody (10 mg) was dissolved in 1 mL of 10 mM PBS, pH 7.4. To this solution was added 22.2 μL of a freshly prepared solution of 20 mg/mL NHS-LC-biotin (Pierce, Rockford, Ill.) in PBS. The vial was wrapped in foil to protect from light and rotated slowly for 30 min at room temperature. The solution was then dialyzed overnight in the dark against 4 L of 20 mM ammonium acetate at 40° C. Aliquots of 0.5 mg biotinylated antibody were transferred to amber vials. To each vial was added 0.5 mL of a solution containing 6 mg/mL each of radioimmunoassay-grade BSA and gelatin. The vials were then lyophilized and stored at 40° C. Individual vials were reconstituted with 0.5 mL of Superfreeze Conjugate StabilizerTM (Pierce) before use. The reconstituted product was stored at −200° C.

[0068] Assay

[0069] Microtiter plates (Immulon-4, Dynatech Laboratories, Chantilly, Va.) were coated overnight at 40° C. with 100 μL/well of a solution containing 4 ug/ml (BoNT A and B), 5 μg/mL (BoNT E) or 2.5 μg/mL (BoNT F) affinity-purified antibody in coating buffer (0.1 M Na_2CO_3, pH 9.6). The remaining sites of absorption were blocked by adding 150 μL/well of Superblock™ blocking reagent (Pierce) for 30 min at 37° C. and then were washed four times with wash buffer (PBS/0.05% Tween 20). Standard curves were prepared by diluting BoNT stock solutions with the appropriate volumes of assay buffer (60 mM PBS, 0.1% BSA, 0.1% Tween 20, and 0.01% sodium azide) or the appropriate human serum matrix. Standards and unknowns (100 μL/well) were incubated for at least 1 hr at 37° C. and the plates washed as above. The appropriate dilution of biotinylated antibody in assay buffer (1:200 for BoNT-A, 1:150 for BoNT-B, 1:250 for BoNT E, 1:400 for BoNT F, 100 μL/well) was added and the plates again were incubated and washed as above. Neutravidin-linked alkaline phosphatase (Pierce, Rockford, Ill., 2 mg/mL in PBS) diluted in assay buffer (1:2,000 for BoNT-A, 1:2,800 for BoNT-B, 1:2,800

for BoNT E, 1:1,500 for BoNT F, 100 μL/well) was then added and the plates were incubated 30 min at 37° C. After a final wash, substrate (para-nitrophenyl phosphate, 1 mg/mL in 1 M Tris, 0.03% MgCl2, pH 9.8) was then added (100 μL/well) and the color allowed to develop for 20-30 min. The optical density at 405 nm was then read on a Bio-Tek 311 Microtiter Plate Reader (Bio-Tek Instruments, Winooski, Vt.). Standard curves were constructed by plotting the absorbance values (mean of triplicate wells) against toxin concentrations, and unknown concentrations were determined from the linear regression equation.

EXAMPLE 1

[0070] Standard curves: background, linearity, and detection limits

[0071] Affinity-purifying the horse anti-BoNT sera resulted in a 10-fold increase in specific activity, as measured by mouse neutralization assay (data not shown). The combined effects of highly-purified capture antibody plus exquisite specificity and affinity of the neutravidin/biotin linkage used to couple the chromogenic enzyme to the biotinylated secondary antibody resulted in low background and high specific absorbance. Background in each assay was typically 0.01-0.02 absorbance units or less (data not shown) and was not subtracted from standard curves. For serotypes A and B, standard curves were linear over the range of 0.1-10 ng/mL (10 pg-1 ng/well) and did not differ significantly between assay buffer or 10% human serum (**FIGS. 1A and 1B**). The detection limit of each assay as described is approximately 0.2 ng/mL (20 pg/well), where absorbance readings were typically twice background. Accurate quantitation was possible at about 0.5 ng/mL (50 pg/well), where absorbance readings were typically 3-5 times background and variations between triplicate wells typically 5-10%. Linearity, as measured by the correlation coefficient (r^2) of the regressed line, ranged from 0.993-0.999 for all assays.

[0072] Background in the BoNT E assay was very low, typically 0.01 absorbance units or less (data not shown). Background in the BoNT F assay was higher, typically 0.1-0.2 absorbance units. The reason for this is unknown, but we were unable to reduce this background by extensively optimizing reagent concentrations without also reducing the slope of the standard curve. It is possible that there was some kind of low-level cross-reactivity of the anti-BoNT F antibody with the blocking reagents, but substituting gelatin, BSA or casein for the SuperblockTM did not appreciably lower the background. In neither assay was the background subtracted from the standard curves. For both serotypes, standard curves were linear over the operating range of the assay and did not differ significantly between assay buffer or 10% human serum (**FIGS. 2A and 2B**). The limit of accurate quantitation for each assay as described was approximately 0.5 ng/mL (50 pg/well) for BoNT E. Due to the higher background, the quantitation limit for BoNT F was 2 ng/mL (200 pg/well). Variation between triplicate wells was typically 5-10%. Linearity, as measured by the correlation coefficient (r^2) of the regressed line, ranged from 0.994-0.997 for all assays.

EXAMPLE 2

[0073] Inter- and intra-assay variation

[0074] To measure inter- and intra-assay variation (repeatability and reproducibility), assay buffer and 10% human serum in assay buffer were spiked with purified BoNT at three concentrations within the standard curve. These solutions were divided into aliquots and kept frozen at -200° C. Aliquots were thawed immediately before analysis.

[0075] To measure intra-assay variability (reproducibility), five assays were performed in a single day. Separate standard curves were prepared independently from the stock solution for each assay. The results of this experiment for BoNT A and B are shown in Table 1. Both accuracy and precision were excellent; deviation from the expected values was 0-5%, and the standard deviations were typically 2-6% of the mean. The results of this experiment for BoNT E and F are shown in Table 2. Both accuracy and precision were again excellent; deviation from the expected values was 0-2.5%, and the standard deviations were typically 2-5% of the mean.

[0076] To measure inter-assay variability (repeatability), one assay was performed on 5 consecutive days. The results of this experiment with BoNT A and B are shown in Table 3 and for BoNT E and F in Table 4. Again, both accuracy and precision were excellent. Deviation from the expected values and standard deviations were similar to those in the intra-assay variation experiment.

TABLE 1

Intra-assay variability (reproducibility) of the BoNT ELISA - s. Five independent assays were performed by the same operator in a single day. Separate standard curves were prepared for each assay. Results are expressed as ng/mL (±SD). Analytes were the appropriate media spiked with purified BoNT as described in the text. Correlation coefficients (r^2) describe the linear regression lines fitted to the standard curves.

	0.5 ng/mL	2.5 ng/mL	8.0 ng/mL	mean r^2
BoNT A (assay buffer)	0.5 (±.03)	2.5 (±.17)	8.4 (±.58)	.997 (±.002)
BoNT A (10% serum)	0.5 (±.01)	2.5 (±.10)	8.2 (±.32)	.995 (±.002)
BoNT B (assay buffer)	0.5 (±.02)	2.6 (±.13)	8.4 (±.53)	.997 (±.001)
BoNT B (10% serum)	0.5 (±.01)	2.5 (±.07)	8.0 (±.24)	.997 (±.002)

[0077]

TABLE 2

Intra-assay variability (reproducibility) of the BoNT ELISAs. Five independent assays were performed by the same operator in a single day. Separate standard curves were prepared for each assay. Results are expressed as ng/mL (±SD). Analytes were the appropriate media spiked with purified BoNT as described in the text. Correlation coefficients (r^2) describe the linear regression lines fitted to the standard curves.

	0.5 ng/mL	2.5 ng/mL	8.0 ng/mL	mean r^2
BoNT E (assay buffer)	0.5 (±0.03)	2.6 (±0.13)	7.9 (±0.17)	0.997 (±0.001)
BoNT E (10% serum)	0.5 (±0.03)	2.5 (±0.05)	7.8 (±0.32)	0.996 (±.002)
	4.0 ng/mL	10.0 ng/mL	16.0 ng/mL	mean r^2
BoNT F (assay buffer)	4.2 (±0.04)	10.1 (±0.07)	15.7 (±0.22)	0.994 (±0.002)
BoNT F (10% serum)	4.1 (±0.06)	10.2 (±0.04)	16.0 (±0.17)	0.994 (±0.003)

[0078]

TABLE 3

Inter-assay variability (repeatability) of the BoNT ELISA = s. Five independent assays were performed on separate days by the same operator. Results are expressed as ng/mL (±SD). Analytes were the appropriate media spiked with purified BoNT as described in the text. Correlation coefficients (r^2) describe the linear regression lines fitted to the standard curves.

	0.5 ng/mL	2.5 ng/mL	8.0 ng/mL	mean r^2
BoNT A (assay buffer)	0.5 (±.02)	2.5 (±.17)	8.5 (±.18)	.998 (±.002)
BoNT A (10% serum)	0.5 (±.01)	2.5 (±.09)	7.8 (±.17)	.998 (±.002)
BoNT B (assay buffer)	0.5 (±.05)	2.4 (±.11)	8.3 (±.49)	.997 (±.001)
BoNT B (10% serum)	0.5 (±.02)	2.5 (±.09)	7.9 (±.45)	.996 (±.002)

[0079]

TABLE 4

Inter-assay variability (repeatability) of the BoNT ELISAs. Five independent assays were performed on separate days by the same operator. Results are expressed as ng/mL (±SD) Analytes were the appropriate medium spiked with purified BoNT as described in the text. Correlation coefficients (r^2) describe the linear regression lines fitted to the standard curves.

	0.5 ng/mL	2.5 ng/mL	8.0 ng/mL	mean r^2
BoNT E (assay buffer)	0.5 (±.02)	2.4 (±.08)	7.8 (±.18)	.997 (±.001)
BoNT E (10% serum)	0.5 (±.02)	2.5 (±.05)	7.9 (±.30)	.995 (±.001)
	4.0 ng/mL	10.0 ng/mL	16.0 ng/mL	mean r^2
BoNT F (assay buffer)	4.2 (±.05)	10.0 (±.09)	15.7 (±.20)	.996 (±.002)
BoNT F (10% serum)	4.2 (±.05)	10.1 (±.12)	16.0 (±.09)	.997 (±.001)

EXAMPLE 3

[0080] Cross-reactivity between serotypes

[0081] BoNT serotypes exhibit 30-60% sequence identity (Oguma et al., 1995, Microbiol. Immunol. 39, 161-168; Singh et al., 1996, Toxicon 34, 267-275). However, serotype-specific antisera have been reported to elicit little or no cross-reactivity (Sakaguchi, 1983, Pharmac. Ther. 19, 165-194; Kozaki et al., 1989, In: Simpson, L. L. (Ed.), Botulinum Neurotoxins and Tetanus Toxin. Academic Press, San Diego, pp. 301-818). The antibodies used in these assays were affinity-purified against the 50 kD C-fragment of the heavy chains of BoNT A, B, E and F. Each assay was <1% cross-reactive against the other serotype and also serotypes A and B (data not shown), suggesting that most common epitopes among serotypes must reside elsewhere on the protein chains.

EXAMPLE 4

[0082] Recognition of toxins with associated nontoxic proteins

[0083] These assays were developed with toxin standards comprised of highly purified toxins devoid of associated proteins. Naturally occurring toxins, however, are typically complexed with NAPs which serve to protect the toxins from acidic and/or proteolytic degradation in the gastrointestinal tract. These proteins could conceivably block antigenic sites and prevent recognition of the toxins by the antibodies. Therefore, we evaluated whether these assays could detect native BoNTs with their naturally occurring NAPs.

[0084] Because NAPs can vary in both molecular weight and in mass ratio to the purified toxin, it was impossible to compare complexed toxins to the purified toxins on a mass basis. To avoid this problem, we used activity units (mouse LD_{50}) as our method of direct comparison. The results of this experiment are shown in **FIGS. 3A and 3B** for BoNT A and B and **FIGS. 4A and 4B** for BoNT E and F. The assays appeared to recognize the complex similarly for BoNT B, and only slightly less than the native toxin (approx 25%) for BoNT A. This amount of variability is consistent with the fact that both toxin preparations in each assay were quantified using the mouse bioassay, where quantitation variability is typically +10%.

[0085] The assay for BoNT F recognized the complex and neurotoxin approximately equally. However, the assay for BoNT E suggested that the antibody recognized the complex significantly better than the purified neurotoxin. These results were counterintuitive because the antibodies were produced against purified neurotoxin, and affinity-purified against purified C-fragment. While complexation with NAPs might easily decrease specific recognition by antibodies due to masking of antigenic determinants, it was difficult to postulate a viable mechanism whereby specific recognition was increased. BoNT E is different than other serotypes. All clostridial neurotoxins are synthesized and expressed as single polypeptide chains of about 1300 aminoacids. Subsequent proteolytic "nicking" produces the heavy and light chains linked by disulfide bridges. This nicking step is required for toxicity. In most serotypes, this proteolytic activation step occurs intracellularly with active toxin being expressed by the bacterial strain involved. With BoNT E, however, no intracellular proteolysis occurs; inactive toxin is expressed by the bacterial culture and extracellular proteolytic activation is required. Commercially available BoNT E is usually artificially "nicked" by mild treatment with trypsin. This step increases toxicity by approximately 10-fold. We postulated that this trypsinization step, in addition to activating the neurotoxin, might also destroy antigenic determinants and render the toxin less recognizable by our antibodies.

[0086] To test this hypothesis, we purchased from our commercial supplier, METAbiologics, Inc., activated (trypsinized) and unactivated BoNT E neurotoxin. We then tested each, along with the complexed form, in the BoNT E ELISA. Results are shown in **FIG. 5**. The complexed toxin and the non-activated toxin were recognized similarly by the assay. Although the minor differences in quantitation may have been real, they are not inconsistent with errors involved in determining activity by mouse bioassay (McLellan et al., 1996, Toxicon 34(9), 975-985). In contrast, the activated neurotoxin was barely recognized at all. This suggests two very important things. First, trypsinizing the purified neurotoxin destroyed critical antigenic determinants required for antibody recognition. Second, the complexed neurotoxin appears to have been largely protected from this effect, while still being effectively activated. Evidently, the NAPs were functioning in the trypsinization step in the same way they function in the gut. That is, they allowed proteases to "nick"

the toxin at the appropriate site(s) for activation, but protected the molecule from further proteolysis that might prove destructive. This information also suggests that the activation step should precede the final purification steps in commercial preparations. That the purified and the complexed toxin should be recognized equally is not unexpected. As for other serotypes (Szylagyi, et al., 2000), the C-fragment of BoNT E is believed to contain regions that are intimately involved with receptor binding (Clayton, et al., 1995). Should the accessibility of these regions be blocked by NAPs, binding would be hindered, with a resulting loss of toxicity. Thus, binding of antibodies purified against the C-fragment is less likely to be sterically hindered by NAPs than that of antibodies directed against the whole toxin or toxoid.

[0087] We present here simple, sensitive and accurate colorimetric capture ELISAs for BoNT neurotoxins type E and F in assay buffer and 10% human serum. These assays demonstrate sensitivities similar to that of the mouse bioassay, yet offer significant savings in both money and time while eliminating the use of animals. Because the antibodies were affinity-purified against the C-fragments of each toxin, interference by NAPs was minimal. In vitro activation of BoNT E by treatment with trypsin destroys antigenic determinants and may be a confounding factor in the development of immunological assays.

What is claimed is:

1. A method for detecting BoNT antigen in a sample comprising:

(a) reacting a sample suspected of having an antigen with a first antibody, Ab(A);

(b) washing the reaction mixture to remove unreacted antibody;

(c) reacting the Ab(A) linked antigen with a second antibody which is conjugated with biotin, Ab(B);

(d) washing the reaction mixture to remove unreacted antibody;

(e) reacting Ab(A)-antigen-Ab(B) with avidin linked to a chromogenic enzyme;

(f) washing the reaction mixture;

(g) reacting the chromogenic enzyme;

(h) measuring optical density of reaction and calculating concentration of antigen using a standard curve.

2. The method of claim 1 wherein said BoNT antigen is chosen from the group consisting of BoNT serotype A, BoNT serotype B, BoNT serotype F, and BoNT serotype E.

3. The method of claim 2 wherein said sample is chosen from the group consisting of: animal serum, food and dirt.

4. The method of claim 3 wherein the standard is assayed simultaneously along with the sample.

5. The method of claim 2 wherein said first antibody is bound to a solid support.

6. The method of claim 5 wherein said solid support is chosen from the group consisting of microtiter plate, membrane, and dipstick.

7. The method of claim 2 wherein said antibody is affinity-purified to heavy chain C-fragment of BoNT.

8. A test kit for the assay of BoNT antigen comprising:

one or more solutions containing a known concentration of free BoNT or complexed BoNT to serve as a standard;

a solution of a anti-BoNT antibody, and biotinylated anti-BoNT antibody affinity purified to the C-fragment of BoNT;

a chromogen which changes color or shade by action with biotin; and, optionally,

a solid support.

9. The kit according to claim 8 wherein said BoNT is chosen from the group consisting of BoNT serotype A, BoNT serotype B, BoNT serotype E, and BoNT serotype F.

10. A test kit of claim 8 wherein said kit further comprises test tubes for said solutions.

* * * * *

US 20020058294A1

(19) **United States**
(12) **Patent Application Publication**
(10) **Pub. No.: US 2002/0058294 A1**
(43) **Pub. Date: May 16, 2002**

Poli et al.

(54) **METHOD FOR DETECTING CLOSTRIDIUM BOTULINUM NEUROTOXIN SEROTYPES A, B, E AND F IN A SAMPLE**

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(21) Appl. No.: **09/952,078**

(22) Filed: **Sep. 14, 2001**

Related U.S. Application Data

(63) Non-provisional of provisional application No. 60/232,929, filed on Sep. 15, 2000.

Publication Classification

(51) **Int. Cl.**[7] **G01N 33/554**; G01N 33/569; G01N 33/53

(52) **U.S. Cl.** **435/7.32**; 435/7.5

(57) **ABSTRACT**

Sensitive and specific enzyme-linked immunosorbent assays which detect Clostridium botulinum neurotoxins serotypes A, B, E, and F in a sample are described. The assay is based upon affinity-purified antibodies directed against the C-fragments of each toxin. These assays demonstrate sensitivity close to that of the mouse bioassay without the use of animals and in a much simpler format than other assays of similar sensitivity.

L5: Entry 23 of 42 File: USPT Apr 4, 2006

DOCUMENT-IDENTIFIER: US 7023544 B2
TITLE: Method and instrument for detecting biomolecular interactions

PRIOR-PUBLICATION:

DOC-ID	DATE
US 20030059855 A1	March 27, 2003

Other Reference Publication (64):
Mullaney, et al., "Epitope Mapping of Neutralizing Botulinum Neurotoxin A Antibodies by Phage Display", Infection and Immunity, vol. 69, No. 10, pp. 6511-6514, 2001. cited by other

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#56	**Related Articles** for **PubMed** (Select **9619584**) sort by: **PublicationDate**	16:15:51	
#12	Search **botulinum peptide** Sort by: **PublicationDate**	14:19:25	10
#11	Search **kazim 1982 atassi**	12:54:13	
#10	Search **kazim 1980 atassi**	12:53:15	
#9	Search **bixler atassi 1984**	12:52:13	
#8	Search **bixler 1984**	12:52:03	
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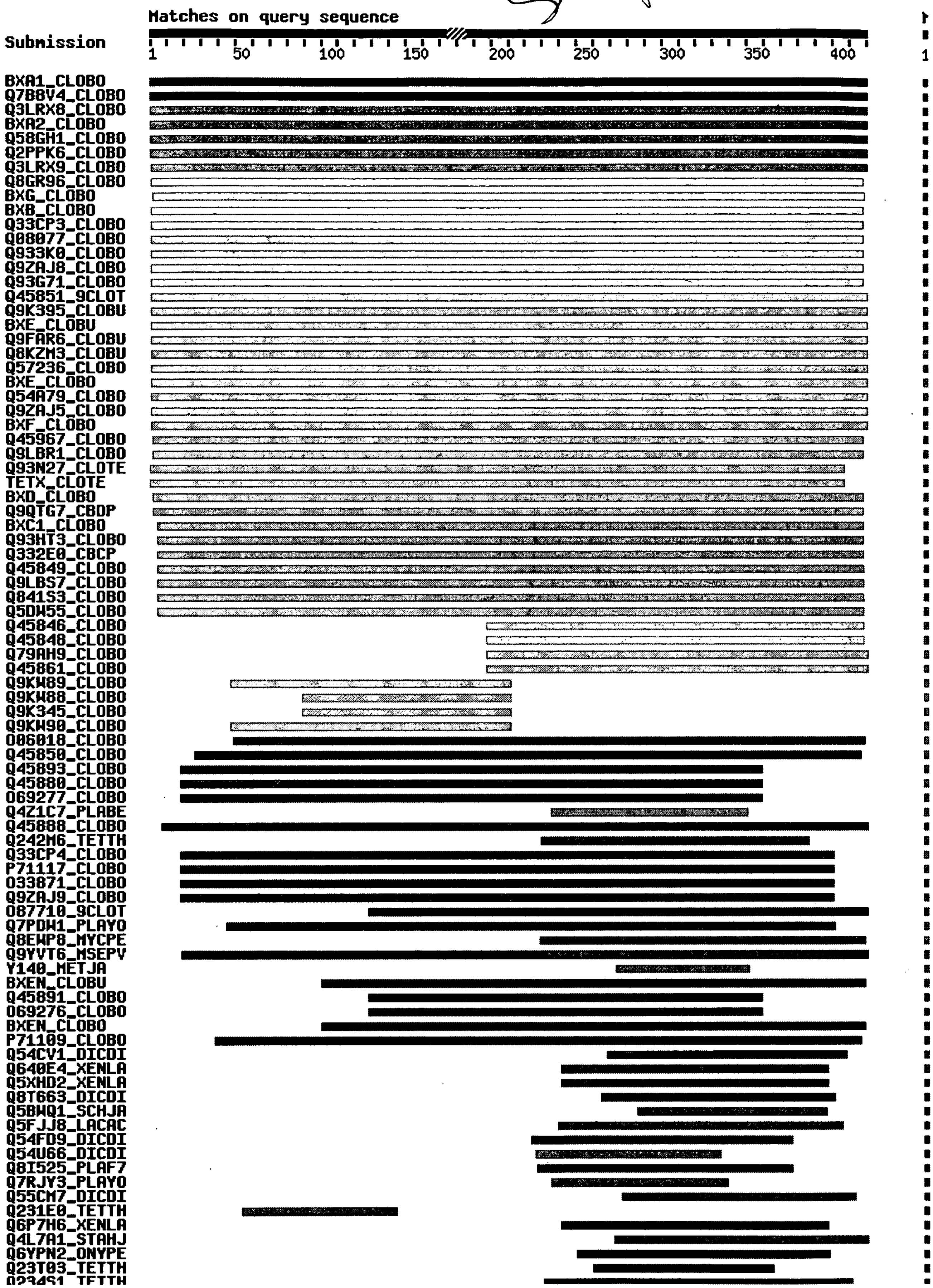

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- ☐ sp Q45894 BXA2_CLOBO Botulinum neurotoxin type A precursor (EC 3.
- ☐ tr Q58GH1 _CLOBO Type A2 botulinum neurotoxin [Clostridium botuli
- ☐ tr Q3LRX8 _CLOBO Neurotoxin type A [Clostridium botulinum]
- ☐ tr Q2PPK6 _CLOBO Botulinum neurotoxin type A [boNT/A] [Clostridiu
- ☐ tr Q3LRX9 _CLOBO Neurotoxin


Profile hits
Pfam hits
Toxin_trans

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ef    Items    RT  Index-term
E1        0        *BOTULINUM TOXIN
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E4      302         BOTULINUM TOXIN TYPE A --ADVERSE EFFECTS --AE
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E6       33         BOTULINUM TOXIN TYPE A --ANTAGONISTS AND INHIB
E7        9         BOTULINUM TOXIN TYPE A --BIOSYNTHESIS --BI
E8        5         BOTULINUM TOXIN TYPE A --BLOOD --BL
E9        3         BOTULINUM TOXIN TYPE A --CHEMICAL SYNTHESIS --
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E35      343         BOTULINUM TOXINS --ADVERSE EFFECTS --AE
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R9      629    B    28   ANTI-DYSKINESIA AGENTS
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S33	78	S32 AND (H2 OR HEAVY OR TRANSLOCAT? OR CHANNEL? OR PORE?)

? logoff hold

1: Immunol Invest. 2002 Aug-Nov;31(3-4):247-62. Related Articles, Links

Cross reaction of tetanus and botulinum neurotoxins A and B and the boosting effect of botulinum neurotoxins A and B on a primary anti-tetanus antibody response.

Dolimbek BZ, Jankovic J, Atassi MZ.

Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, Texas, USA.

The present studies were carried out in order to investigate the cross-reaction of botulinum neurotoxins (BoNTs) with human and mouse antibodies against tetanus neurotoxin (TeNT) and determine whether injection of BoNT into a host that has been primed with TeNT would result in boosting of the response to the injected BoNT. Human antisera against TeNT obtained from 9 individuals were found to exhibit substantial cross-reaction with BoNTs A and B. We prepared antibodies (Abs) against inactivated tetanus neurotoxin (TeNT) in outbred mice and determined the binding of these Abs to active TeNT and active botulinum neurotoxins (BoNTs) A and B. Blood samples were collected before immunization (day 0) and on days 42, 82 and 125 after the first injection. The reactions of these sera with the immunizing antigen (inactivated TeNT), active TeNT, active BoNT/A and active BoNT/B were determined. At a fixed dilution (1:62.5 v/v), the sera contained high levels of Abs that reacted with TeNT and also with BoNTs A and B. Throughout the test period (up through day 125) and at different dilutions the cross-reactions of the antisera with BoNT/B were almost twice those with BoNT/A. The reactions of the antisera with the immunizing antigen (inactive TeNT) or with active TeNT were essentially equal throughout the dilution range tested (1:16-1:500 v/v). To determine whether injection of BoNT/A or B into a host that had been primed with TeNT resulted in boosting of the response to the priming antigen (TeNT) as well as BoNT/A or B, mice were primed with TeNT and boosted 21 days later with TeNT, BoNT/A or BoNT/B. Appropriate controls were also employed. Blood samples were collected prior to TeNT priming (day -1) and on days 21, 32, 46 and 67 after priming. In TeNT-primed mice, BoNTs A or B boosted the anti-TeNT Ab responses slightly but had no significant boosting effect on the Ab populations that bind to BoNTs A or B. It is concluded that while Abs against TeNT cross react with BoNTs and the cross reaction with BoNT/B is almost double that of BoNT/A, injection of BoNTs A or B in the presence of a prior active immunity against TeNT is not very likely to make the host mount an Ab response against the injected BoNT.

PMID: 12472183 [PubMed - indexed for MEDLINE]

further comprise separating the BoNT/A from the antibody and recovering the BoNT/A. In one embodiment, the separation is conducted by contacting the immunological complex with a saturating amount of peptide comprising the epitope recognized by the antibody of the immunological complex.

Summary of Invention Paragraph:
[0007] The present invention still further pertains to a method for detecting BoNT/A in a sample. The method involves contacting the sample with an antibody which binds an epitope of BoNT/A, allowing the antibody to bind to BoNT/A to form an immunological complex, and detecting the formation of the immunological complex and correlating presence or absence of the immunological complex with presence or absence of BoNT/A in the sample. The sample can be biological, environmental, or a food sample.

Summary of Invention Paragraph:
[0008] Yet another aspect of the present invention is a kit for detecting BoNT/A in a sample. The kit includes a container holding an antibody which binds to an epitope of BoNT/A and instructions for using the antibody for the purpose of binding to BoNT/A to form an immunological complex and detecting the formation of the immunological complex such that presence or absence of the immunological complex correlates with presence or absence of BoNT/A.

Brief Description of Drawings Paragraph:
[0015] FIG. 4. The MAb 6B2-2 binds to a distinct epitope on BoNT/A. Epitope mapping of the MAbs was carried out by SPR, Affinity-purified antibody to mouse IgG Fc was immobilized onto the chip. Purified-BoNT/A HC MAb was captured by the antibody, and then nonspecific sites were blocked by passing a saturating concentration of an unrelated antibody over the matrix surface. BoNT/A HC (200 nM) in HEPES buffered saline was passed over the antibodies at a flow rate of 5 .mu.l/min. Finally, the second (competing) MAb was injected, and its binding determined. The biosensor chip was regenerated and the process was repeated to test the ability of all MAbs to bind as second MAb using each MAb as first MoAb.

Detail Description Paragraph:
[0021] The present invention provides antibodies which bind to epitopes of BoNT/A and BoNT/A Hc. These antibodies can be used to purify BoNT/A from an impure solution containing BoNT/A, and to detect BoNT/A in by a sample and as a treatment for BoNT/Z intoxication. In addition, the antibodies can be used in kits for using in the methods described.

Detail Description Paragraph:
[0027] The term "epitope" is art-recognized. It is generally understood by those of skill in the art to refer to the region of an antigen, such as BoNT/A, that interacts with an antibody. An epitope of a peptide or protein antigen can be formed by contiguous or noncontinguous amino acid sequences of the antigen. BoNT/A, like many large proteins, contains many epitopes. Examples of BoNT/A epitopes recognized by antibodies of the present invention include the amino acid sequences 1150-1289 of BoNT/A Hc (SEQ ID NO:1), amino acids 1157-1181 (SEQ ID NO:2), and amino acids 1230-1253 (SEQ ID NO:3). These peptides offer a convenient method for eluting BoNT/A bound to either MAb 4A2-2, 4A2-4, 6E9-3, 6E9-4, 6E10-4, 6E10-5, 6E10-8, 6E10-10, 6B2-2, and 6C2-4 on immunoaffinity columns. For example, when an antibody which recognizes the epitope for either MAb 4A2-2, MAb 6C2-4, or MAb 6B2-2, is used in an immunoaffinity column to purify BoNT/A, the peptide recognized by the antibody can be added to the immunoaffinity column to elute the BoNT/A. See below for a more detailed description of the purification of BoNT/A.

Detail Description Paragraph:
[0028] Epitope mapping studies described in this application defined three groups of MAbs, corresponding to two-distinct and one overlapping protective-epitope regions on BoNT/A Hc. One particular region of the antigen was defined by MAb 6B2-

2, while 4A2-2, 4A2-4, 6E10-5, 6E10-8, 6E10-10, 6E9-3, 6E9-4, 6E9-12, and 6E10-4 MAbs bound a distinct site. The MAb 6C2-4 defined a site that overlaps with 6E10-5, 6E10-8, 6E10-10, 6E9-3, 6E9-4, 6E9-12, and 6E10-4 MAbs. However, 6C2-4 MAb bound an epitope that is distinct from 4A2-2 and 4A2-4 binding site. Thus, based on this analysis, three possible neutralizing epitopes are recognized by the MAbs of the present invention.

Detail Description Paragraph:
[0029] The epitopes to which the monoclonal antibodies bind on BoNT/A have been identified as one of the principal protective antigenic determinants of BoNT/A, suggesting that an eventual vaccine candidate may include several of these epitopes or peptides containing these antigenic determinant. The peptides were selected based on a secondary structure prediction algorithm (Rost and Sander 1994, Protein Struct. Funct. Gen. 20, 216-226) that located highly solvent exposed residues of BoNT/A. These highly exposed areas are postulated to be sites of interaction between antibodies and BoNT/A. We have identified two peptides described in SEQ ID NO:2 and SEQ ID NO:3 and based on binding of neutralizing MAbs to these areas, we believe that these two peptides are a part of a single protective epitope on the Hc of BoNT/A.

Detail Description Paragraph:
[0032] The present invention also pertains to hybridomas producing antibodies which bind to an epitope of BoNT/A. The term "hybridoma" is art recognized and is understood by those of ordinary skill in the art to refer to a cell produced by the fusion of an antibody-producing cell and an immortal cell, e.g. a multiple myeloma cell. This hybrid cell is capable of producing a continuous supply of antibody. See the definition of "monoclonal antibody" above and the Examples below for a more detailed description of the method of fusion. The hybridoma which produces MAb 4A2-2 is deposited under ATCC Accession Number PTA-971. The hybridoma which produces MAb 6B2-2 is deposited under ATCC Accession Number PTA-969. The hybridoma which produces MAb 6C2-4 is deposited under ATCC Accession Number PTA-970.

Detail Description Paragraph:
[0033] The present invention further pertains to a method for purifying BoNT/A from an impure solution containing BoNT/A. The method involves contacting the impure solution with an antibody which binds an epitope of BoNT/A, allowing the antibody to form an immunological complex, and separating the complex from the impure solution. This method can be used to clear toxic amounts of BoNT/A from any biological fluid and subjects including animals and humans.

Detail Description Paragraph:
[0034] The method of purification can further comprise separating the BoNT/A from the antibody and recovering the BoNT/A. In one embodiment, the separation is conducted by contacting the immunological complex with a saturating amount of peptide comprising the epitope recognized by the antibody of the immunological complex.

Detail Description Paragraph:
[0040] The present invention still further pertains to a method for detecting BoNT/A in a sample containing BoNT/A. The method includes contacting the sample with an antibody which binds an epitope of BoNT/A, allowing the antibody to bind to BoNT/A to form an immunological complex, and detecting the formation of the immunological complex and correlating presence or absence of the immunological complex with presence or absence of BoNT/A in the sample. The sample can be biological, environmental or a food sample.

Detail Description Paragraph:
[0045] Yet another aspect of the present invention is a kit for detecting BoNT/A in a biological sample containing BoNT/A. The kit includes a container holding an antibody which binds an epitope of BoNT/A and instructions for using the antibody

for the purpose of binding to BoNT/A to form an immunological complex and detecting the formation of the immunological complex such that the presence or absence of the immunological complex correlates with presence or absence of BoNT/A in the sample. Examples of containers include multiwell plates which allow simultaneous detection of BoNT/A in multiple samples.

Detail Description Paragraph:
[0078] Epitope mapping of the MAbs was carried out by surface plasmon resonance at a flow rate 5 .mu.l/min. Affinity-purified antibody to mouse IgG Fc was immobilized onto the chip, purified BoNT/A HC MAb was captured by the antibody, and then nonspecific sites were blocked by passing a saturating concentration of an unrelated antibody over the matrix surface. BoNT/A HC (200 nM) in HEPES buffered saline was passed over the antibodies at a flow rate of 5 .mu.l/min. Finally, the second (competing) MAb was injected, and its binding determined. The biosensor chip was regenerated as above, and the process was repeated to test the ability of all MAbs to bind as second MAb using each MAb as first MoAb. Thus, all antibody pairs were tested in both directions.

Detail Description Paragraph:
[0088] Next, we used SPR to characterize the binding sites of the Abs. In these studies, we used anti-mouse antibody to capture the BoNT/A Hc MAb, followed by BoNT/A Hc. A second competing MAb, was injected, and its was binding determined. This experiment was repeated to examine the ability of all MAbs to bind as second MAb using each MAb as first MAb. This type of approach tested all pairs of antibodies in both directions. As seen in an example in FIG. 4, when 4A2-2 MAb was immobilized onto the sensor chip and the same antibody or other heterologous antibody, 6B2-2, 6E9-1, and 6E10-8, respectively, were injected sequentially, only competing MAb 6B2-2 bound. All combination of antibodies was tested likewise and the data obtained are summarized in FIG. 5. The epitope mapping studies defined three groups of MAbs, corresponding to two-distict and one overlapping protective-epitope regions on BoNT/A Hc. One particular region of the antigen was defeined by MAb 6B2-2, while 4A2-2, 4A2-4, 6E10-5, 6E10-8, 6E10-10, 6E9-3, 6E9-4, 6E9-12, and 6E10-4 MAbs bound a distinct site. The MAb 6C.sub.2-4 defined a site that overlaps with 6E10-5, 6E10-8, 6E10-10, 6E9-3, 6E9 4, 6E9-12, and 6E10-4 MoAbs. However, 6C.sub.2-4 MAb bound an epitope that is distinct from 4A2-2 and 4A2-4 MoAb binding site. Thus, based on this analysis, we are proposing that there are at leat two, possibly three, neutralizing epitopes on BoNT/A Hc.

Detail Description Paragraph:
[0089] Immunization with pentavalent toxoid (composed of BoNT serotypes A-E) or H.sub.c of BoNT/A produced high antibody titers against BoNT/A and protected mice against 10, 100, and 1000 LD.sub.50 of BoNT/A (Table 3). However, when mice were vaccinated with H.sub.c of BoNT serotype B or E, little to no antibody against BoNT/A was detected. These mice were not protected when challenged with 10 LD.sub.50 BoNT/A. This experiment suggests there was little to no cross-protection among these serotypes, and that the majority of protective epitopes of BoNT/A was located within Hc (Dertzbaugh and West, 1996, supra; Clayton et al., 1995, Infect. Immun. 63, 2738-2742).

Detail Description Paragraph:
[0090] To identify the sites that contain the neutralizing epitopes, protective MAbs to the Hc of BoNT/A were produced in mice.

Detail Description Paragraph:
[0091] To characterize the region of BoNT/A H.sub.c that these MAbs recognized, the MAbs were reacted separately to each of five recombinant overlapping fragments encompassing H.sub.c of BoNT/A-Hc (Dertzbaugh and West, 1996, supra). As expected, MAbs were able to immunoprecipitate H.sub.c of BoNT/A (-50 kDa) (FIG. 6). The MAbs, 6E9-12, 4A2-2, and 6C2-2, immunoprecipitated the fragment corresponding to residues 1150-1289. However, none of the MAbs recognized the truncated fragment

corresponding to the N-terminal portion of BoNT/A H.sub.c amino acid residues 915-1059. Although the other fragments were not recognized, or were only recognized weakly, by the MAbs, they were recognized by polyclonal antibodies produced to BoNT/A H.sub.c, which suggested that they may contain other B-cell epitopes (data not shown). Control MAb (SEB-2Ag) produced to a bacterial superantigen (Ulrich et al., 1995, Trends Microbiol. 3, 463-468), staphylococcal enterotoxin B (SEB), did not immunoprecipitate any of the fragments. Of the fragments derived from BoNT/A H.sub.c, only immunization with the 1150-1289 peptide, which contained the residues within the carboxyl-terminal end of the BoNT/A H.sub.c, protected mice from BoNT/A challenge (Dertzbaugh and West, 1996, supra). Together, these data strongly suggest that the majority of PPDs of BoNT/A reside within the carboxyl-terminal end of the molecule.

Detail Description Paragraph:
[0093] Because recognition of a peptide by neutralizing MAbs is a prerequisite for the presence of PPADs, we examined binding of the MAbs to engineered synthetic peptides. The antibodies were incubated with biotin-labelled peptides, and then the complexes were captured on the wells of ELISA plates by anti-IgG Fc antibodies. Bound peptides in complex with the neutralizing MAb were detected using streptavidin. The neutralizing MAbs recognized both peptides that were predicted to be highly exposed (FIGS. 8a,b), while the control peptide was not recognized (FIG. 8c). Although both peptides were similarly highly exposed, peptide 2 was better recognized than peptide 1 by the MAbs. We failed to detect any interaction between the peptides and anti-SEB MAb (SEB-2Ag) (FIG. 8). Because all of the MAbs examined recognized both H.sub.c-peptides, it is likely that these spatially proximal peptides correspond to a single neutralizing epitope on the Hc of BoNT/A.

Detail Description Paragraph:
[0095] Taken together, the results so far suggested that the epitopes detected by MAbs were good immunogens and, therefore, the peptides were used as candidates in a preliminary vaccine trial. Mice were immunized with a single peptide, combination of peptides, or BoNT/A H.sub.c, and challenged 3 weeks after the final immunization with BoNT/A (Table 5). The highest dose was most effective in eliciting high antibody titers for all peptides (data not shown). All mice that were vaccinated with 2 or 10 .mu.g of peptide and challenged with 10 LD.sub.50 of BoNT/A died. Mice immunized with peptide 1 were not protected against any lethal challenge doses. Vaccination with peptide 2 resulted in 40% survival when mice were given 10 or 40 .mu.g and challenged with 5 or 10 LD.sub.50, respectively. Slightly more protection was afforded when combination of peptide 1 and 2 were used in the vaccination protocol. This finding is consistent with the hypothesis that these two peptides may be a part of a single protective epitope on the Hc of BoNT/A. All mice vaccinated with BoNT/A H.sub.c were completely protected against challenge. Mice that received control peptide, then challenged with 5 or 10 LD.sub.50 of BoNT/A were all killed. No noticeable effects on the outcome of these experiments were observed when peptides were conjugated to KLH, or administered with other adjuvants (data not shown).

Detail Description Paragraph:
[0097] There is some evidence from this study and previously published data that significant discontinuity may exist in neutralizing epitopes within BoNT/A (Dertzbaugh and West, 1996, supra). In addition, epitope mapping of these MAbs by using a constrained-peptide display library supports the existence of discontinuous epitopes within BoNT/A (M. Segall and S. Bavari, unpublished data). Therefore, we believe a single, short-peptide vaccine may not be feasible for generating protective immunity against BoNT/A. However, because a single MAb with a very high affinity can block the lethality of BoNT/A, it might be possible to design a vaccine with a combination of two peptides.

CLAIMS:

7. A monoclonal antibody which binds an epitope comprising amino acids 1150-1289 of BoNT/A.

8. A monoclonal antibody which binds an epitope comprising amino acids 1157-1181 of BoNT/A.

9. A monoclonal antibody which binds an epitope comprising amino acids 1230-1253 of BoNT/A.

10. A monoclonal antibody which binds an epitope comprising 1157-1253 of BoNT/A.

22. A vaccine for BoNT/A comprising antigenic peptide epitopes recognized by at least one monoclonal antibody selected from the group consisting of 4A2-2, 6B2-2, and 6C2-2.

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L5: Entry 11 of 42 File: PGPB Jun 10, 2004

DOCUMENT-IDENTIFIER: US 20040110284 A1
TITLE: Antibodies against type a botulinum neurotoxin

Abstract Paragraph:
Antibodies for binding epitopes of BoNT/A and hybridomas which produce such antibodies are described. The antibodies of the present invention can be used in a method for detecting BoNT/A in a sample and/or in a method for purifying BoNT/A from an impure solution. In addition, the antibodies can be used for passive immunization against BoNT/A intoxication or as intoxication therapy. Another aspect of the invention is a kit for detecting BoNT/A in a sample.

Summary of Invention Paragraph:
[0004] Probably due to unusually high toxicity of BoNTs, previous attempts to produce large numbers of high affinity neutralizing monoclonal antibodies (MAbs) against these neurotoxins have been unsuccesful. We reasoned because immunization with non-toxic binding fragment of BoNT/A can induce protective immunity in mice, then it should be possible to generate neutralizing antibodies using this approach. We report herein that immunization with BoNT/A-Hc allowed the generation of MAbs recognizing both the whole BoNT/A and BoNT/A Hc. We characterized these antibodies in detail, demonstrated biochemical detection of BoNT/A and its binding fragment. We used, neutralizing MAbs directed against the BoNT/A-Hc, in combination with theoretically derived predictions of secondary and solvent accessibility of the residues within the BoNT/A-Hc, to locate the principle protective antigenic determinants (PPDs) of BoNT/A-Hc. Binding of the neutralizing MAbs to overlapping truncated recombinant polypeptides corresponding to BoNT/A-Hc were examined. In addition, we tested MAb recognition of two synthetic 25-mer peptides, whose sequences correspond to predicted solvent-exposed loops within the C-terminal end of the BoNT/A-Hc. Finally, we examined the ability of these peptides to elicit antibody production and to determine whether the resultant antibodies protected the immunized mice from BoNT/A challenge. From these experiments, we identified two regions within the Hc that may contribute to a neutralizing epitope. Because of their ability to neutralize BoNT/A, they could be used for mapping binding sites of the toxin, for competitive-based ELISA to predict immunity following vaccination, identify protective epitopes, and they may be important tools for therapeutic purposes.

Summary of Invention Paragraph:
[0005] Therefore, it is one object of the present invention to provide protective antibodies against BoNT/A. The antibodies of the present invention can be monoclonal or polyclonal antibodies. The present invention also pertains to hybridomas producing antibodies, such as 4A2-2, 6B2-2, and 6C2-4, which bind to an epitope of BoNT/A.

Summary of Invention Paragraph:
[0006] It is another object of the present invention to provide a method of purifying BoNT/A from an impure solution containing BoNT/A. The method involves contacting the impure solution with an antibody which binds an epitope of BoNT/A, allowing the antibody to bind to BoNT/A to form an immunological complex, and separating the complex from the impure solution. The method of purification can